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(21) International Application Number: PCT/AU92/00333 (22) International Filing Date: 6 July 1992 (06.07.92) (30) Priority data: PK 7058 4 July 1991 (04.07.91) AU (71) Applicant (for all designated States except US): GARVAN INSTITUTE OF MEDICAL RESEARCH [AU/AU]; St. Vincent's Hospital, 384 Victoria Street, Darlinghurst, NSW 2010 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only) : TSENG, Albert, Peng, Sheng [AU/AU]; 6 Wyvern Street, Epping, NSW 2121 (AU). INGLIS, Adam [AU/AU]; 47 Hillside Parade, Strathmore, VIC 3041 (AU).		(74) Agent: F.B. RICE & CO.; 28A Montague Street, Balmain, NSW 2041 (AU). (81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE). Published With international search report.

(54) Title: PLA2 INHIBITORY COMPOUNDS

(57) Abstract

The present invention provides peptides and compounds which inhibit the enzyme activity of Type II phospholipases A₂. The preferred compounds are pentapeptides. Where the phospholipase human Type II phospholipase A₂ the preferred peptides are FLSYK and KFLSY.

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PLA₂ INHIBITORY COMPOUNDSField of the Invention

The present invention relates to peptides which inhibit the enzymatic activity of phospholipases A₂ (PLA₂s) and illustrated with peptides which inhibit the activity of Type II PLA₂'s particularly synovial PLA₂ and snake PLA₂ (Crotalus durissus and Crotalus atrox). In addition, the present invention relates to pharmaceutical composition including, as the active ingredient these peptides and to methods of treatment involving the administration of this composition.

Background of the Invention

Phospholipases A₂ constitute a diverse family of enzymes with two subclasses (Type I and Type II) (Fig. 1), based on the positions of the disulphide bonds in the molecules while bee venom PLA₂ constitutes a third substantially distinct class of PLA₂. X-ray crystallography has revealed that the segments comprising the functional substructure of the enzyme is similar in classes. This similarity is particularly striking when the structurally-related Type I/II enzymes are compared with bee venom enzyme (2). PLA₂ hydrolyses the sn-2 acyl ester bond of phosphoglycerides initiating the release of fatty acid precursors of inflammatory eicosanoids. Human synovial PLA₂ (a Type II molecule) has recently been isolated and identified (3). The same PLA₂ has been implicated in the pathogenesis of several inflammatory diseases in humans such as rheumatoid arthritis and Gram negative septic shock (7;8).

Murine, inhibitory monoclonal antibodies raised against synovial PLA₂ have demonstrated pre-clinical efficacy. Accordingly, there is interest in the development of compositions which inhibit the enzymatic activity of PLA₂.

Tryptic digestion of human synovial PLA₂ and

- 2 -

subsequent separation and analysis of the fragments by HPLC gave a very interesting and unexpected result for one of the peaks in that it contained two peptides; one a heptapeptide (the N-terminal peptide) and the other a pentapeptide, FLSYK (corresponding to residues 70-74 in other PLA₂ molecules, based on three-dimensional structural "homology" of mammalian PLA₂ amino acid sequences (1,4)). The pentapeptide was found in an earlier eluting, fully resolved peak (as expected). Since the HPLC system failed to fully resolve these two peptides in the latter peak, these data suggest that the two peptides had a strong affinity for one another and raised questions as to the structural implications of this. X-ray diffraction studies (5,6) have shown that amino acid residues in the two peptides are close to the active site of the enzyme and are important in forming or stabilising the channel in which the 1,2-diacyl-3-sn-phosphoglyceride substrate is precisely positioned for hydrolysis of the 2-ester bond. The first turn of the N-terminal helix (residues 1 to 12) is stabilised by a hydrogen bond network provided by the N-terminus and residue 4, elements of residues 69 to 71 and a water mediated link to the catalytic residues; residues 2 and 5 form the "floor" of the channel, residue 9 forms the right wall and the left wall is formed by residue 69 (either tyrosine or lysine usually) which is predicted to move after the substrate has docked and to form a hydrogen bond with the sn-3 phosphate of the substrate. The chemical evidence of the strong interactions between the heptapeptide and the pentapeptide prompted the supposition that the PLA₂ activity may be inhibited in the presence of either one of these peptides.

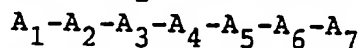
Using synthetic peptide chemistry the present inventors have prepared the pentapeptide FLSYK and demonstrated that addition of it to the assay medium

- 3 -

decreased the enzyme activity of human synovial PLA₂ (Fig 2a). Furthermore, it has been demonstrated that the pentapeptide that occupies the 70-74 region of snake PLA₂ (WDIYR) also inhibited the activity of snake PLA₂ (see Fig. 3b). It is believed that this inhibition is mediated by the peptide binding to the amino terminal end of the enzyme and blocking the reaction either by blocking the substrate access to the hydrophobic channel or by distorting the structure sufficiently to prevent correct orientation of the substrate.

Summary of the Invention

Accordingly, in a first aspect the present invention consists in a linear or cyclic peptide of at least 5 residues which inhibits the enzymatic activity of human synovial PLA₂, the peptide having the following formula:-



in which A₁ is H or one of two naturally occurring amino acids

- A₂ is F or Y or W or absent
- A₃ is L or V or I or M
- A₄ is S or T
- A₅ is Y or F or W
- A₆ is K or R or H or absent
- A₇ is OH or one or two naturally occurring amino acids.

In a preferred embodiment the peptide is a pentapeptide.

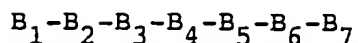
In another preferred embodiment of the present invention A₁ is H and A₇ is OH.

In a further preferred embodiment of the present invention the peptide is FLSYK or KFLSY and most preferably FLSYK.

In a second aspect the present invention consists in a linear or cyclic peptide of at least 5 residues which inhibits the enzymatic activity of crotalus durissus

- 4 -

PLA₂, the peptide having the following formula:-



in which B₁ is H or one of two naturally occurring amino acids

5 B₂ is W or F or Y or absent

B₃ is D or E

B₄ is I or V or L or M

B₅ is Y or F or W

B₆ is R or K or H or absent

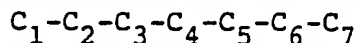
10 B₇ is OH or one or two naturally occurring amino acids.

In a preferred embodiment the peptide is a pentapeptide.

In another preferred embodiment of the present
15 invention B₁ is H and B₇ is OH.

In a further preferred embodiment of the present invention the peptide is WDIYR.

In a third aspect the present invention consists in a linear or cyclic peptide of at least 5 residues which
20 inhibits the enzymatic activity of Crotalus atrox PLA₂, the peptide having the following formula:



in which C₁ is H or one of two naturally occurring amino acids

25 C₂ is T or S or absent

C₃ is V or I or L or M

C₄ is S or T

C₅ is Y or F or W

C₆ is T or S or absent

30 C₇ is OH or one or two naturally occurring amino acids.

In a preferred embodiment the peptide is a pentapeptide.

In another preferred embodiment of this aspect of the
35 present invention C₁ is H and C₇ is OH.

In a further preferred embodiment of this aspect of the present invention the peptide is TVSYT.

As will be clear to those skilled in the art from the disclosure provided herein, the peptides of the first and
5 second aspect of the present invention illustrate how the enzymatic activity of other PLA₂s may be inhibited. This inhibition may be achieved by compounds which interact with the N-terminal amino acid sequence of the PLA₂ molecule in a manner such that the channel into
10 which the phospholipid diffuses prior to catalytic cleavage is destabilized.

Accordingly, in a fourth aspect the present invention consists in a compound which inhibits the enzymatic activity of phospholipase A₂, the compound being
15 characterized in that it interacts with the N-terminal amino acid sequence of the phospholipase A₂ such that the channel into which the phospholipid diffuses prior to catalytic cleavage is either blocked or destabilized.

In a preferred embodiment of the present invention
20 the PLA₂ is human PLA₂ and the compound is a peptide.

In a preferred embodiment of the present invention the peptide has the amino acid sequence FLSYK or KFLSY.

As will be clear to those skilled in the art, the present inventors have found that the enzymatic activity
25 of a phospholipase A₂ can be inhibited by a peptide having a sequence corresponding to a sequence selected from the region of residues 69 to 75 of the phospholipase 2.

Accordingly, in a fifth aspect the present invention
30 consists in a peptide of 5 or 6 residues which inhibits the enzymatic activity of a phospholipase A₂, the peptide having an amino acid sequence corresponding to a sequence selected from the region of residues 69-75 of the phospholipase A₂.

35 In a preferred embodiment this aspect of the present

- 6 -

invention the peptide is a pentapeptide and has an amino acid sequence corresponding to the sequence from residue 69-73 or 70-74 of the phospholipase A₂.

In a further preferred embodiment of the present invention the phospholipase A₂ is human phospholipase A₂.

In a sixth aspect the present invention consists in a composition for use in treating a subject suffering from septic shock rheumatoid arthritis and/or other inflammatory diseases, the composition comprising a therapeutically acceptable amount of peptide or compound of the first, fourth or fifth aspect of the present invention and a pharmaceutical acceptable sterile carrier.

In a seventh aspect the present invention consists in a method of treating septic shock and/or inflammatory disease in a subject comprising administering to the subject the composition of the sixth aspect of the present invention.

It will be appreciated by those skilled in the art that a number of modifications may be made to the peptides of the present invention without deleteriously effecting the biological activity of the peptide. This may be achieved by various changes, such as insertions, deletions and substitutions, either conservative or non-conservative in the peptide sequence where such changes do not substantially decrease the biological activity of the peptide. By conservative substitutions the intended combinations are:-

G, A; V, I, L, M; D, E; N, Q; S, T; K, R, H; and F, Y, W.

It may also be possible to add various groups to the peptide of the present invention to confer advantages such as increased potency or extended half life in vivo, without substantially decreasing the biological activity of the peptide.

It is intended that such modifications to the peptide of the present invention which do not result in a decrease in biological activity are within the scope of the present invention.

5 Detailed Description of the Present Invention

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following examples and Figures, in which:-

10 Fig. 1 shows mammalian PLA₂ amino acid sequences.

Fig. 2: Inhibition of human PLA₂ using the peptide FLSYK.

Fig. 2(a) was obtained using a peptide from a tryptic digest of the enzyme (n=7 ☐ control ☒ inhibitor),
15 2(b) and 2(c) with a synthetic peptide n=11 ☐
control ☒ inhibitor ☐ control ☒ inhibitor,
respectively. The synthetic peptide also inhibits the enzyme in septic shock serum [Fig. 2(c)].

Fig. 3: Dose response curves showing increasing
20 inhibitor with increasing amount of FLSYK and human recombinant Type II PLA₂ (3a ☐ inhibitor, control) and in PLA₂ in septic shock serum (3b ☐ inhibitor ☒ control).

Fig. 4: Dose response curves for FLSYK (4a ☐ PLA₂ ☒ control) and WDIYR (4b ☐ snake (II) ☒ control) on human PLA₂ and snake (Crotalus Durissus) PLA₂ respectively. Both peptides occupy similar sites in their parent proteins and show inhibitory properties for the enzymatic activity.

30 Fig. 5 shows a Lineweaver-Buspe plot showing inhibition of PLA₂ by FLSYK (PLA₂ ☒ 10ug ☒ FLSYK ☐ 1ug FLSYK).

- 8 -

Inhibition of PLA2 ActivityProteins and Peptides

1. Synovial PLA₂, snake PLA₂ (Crotalus Durissus and Crotalus ATR?)
- 5 2. Phe-Leu-Ser-Tyr-Lys (FLSYK)
3. Acetyl-Phe-Leu-Ser-Tyr-Lys-Methyl ester (Ac-FLSYK-OMe)
4. Trp-Asp-Ile-Tyr-Arg (WDIYR)
5. Lys-Phe-Leu-Ser-Tyr (KFLSY)
6. Thr-Val-Ser-Tyr-Thr (TVSTT)
- 10 7. Phe-Lys-Thr-Tyr-Ser (FKTYS)
8. Thr-Glu-Ser-Tyr-Ser (TESYS)
9. Gly-Thr-Lys-Phe-Leu-Ser-Tyr-Lys-Phe-Ser-Asn (GTKFLSYKFSN)
10. Lys-Phe-Leu-Ser-Tyr-Tyr (KFLSYY)
- 15 11. Phe-Leu-Ser-Tyr (FLSY)
12. Phe-Leu-Ser-Tyr-Lys-NH₂. (FLSYK-NH₂)

Tryptic Digestion of PLA2:

- Approximately 100µg of PLA 2 was dissolved in 300µl of 1M Tris pH 8.0 15 µl of Trypsin solution (10µ /1M Tris pH 8) was added and the peptide/trypsin solution was incubated for 2 hours at 37°C. 5µl of neat TFA was used to lower the pH to terminate the digestion. The whole solution was subjected to microbore HPLC fractionation.

25 Microbore HPLC fractionation:

- An ABI Microbore syringe pump system Model 140 was used. Detector wavelength was set at 220nm at 0.5 aufs. A RP-300 1x100mm was used. Fractionation was carried out by running a linear buffer gradient from 0.1% TFA in water to 0.1% TFA, 70% acetonitrile in water over sixty minutes.
- 30 Amino acid sequences identified from fractions were:

- | | |
|-------------|------------|
| Fraction #2 | (K)YQYYSNK |
| Fraction #4 | FLSYK |
| Fraction #5 | FLSYK |
| 35 | NLVNFHR |

- 9 -

Fraction #7* EALLSYGFYG(C)H(C)GVGGR
(C)(C)VTHD(C)(C)YK
SQL(C)E(C)DK
IT(C)AK

5 AAAT(C)FAR

Fraction #9 EAALSYGFYG

*peptides are held together by cystinyl bonds; () denotes tentative assignment.

Peptide Synthesis:

10 Peptide synthesis was carried out in an ABI Peptide Synthesiser Model 430A. T-Boc chemistry was used. HF cleavage was used to release peptide from the solid support.

PLA₂ Serial Dilution:

15 Control: 10 μ l of a standard PLA₂ solution was used at a concentration of 120ng/10 μ l in 20mM Tris pH 8. Serial dilution was done by adding 20mM Tris pH 8 buffer to the final volume of 20 μ l.

20 Inhibitor solution: Pentapeptide was usually dissolved in 1 μ l of 0.1% TFA solution and further 9 μ l of 20mM Tris pH8 was added. This solution was always maintained around pH7-8. 10 μ l of this inhibitor solution was added into 10 μ l of PLA₂ solution.

25 Incubation: all samples were incubated at 37°C for one hour.

PLA₂ solution: A standard PLA 2 solution was prepared in 20mM Tris pH8.0 so that 10 μ l will give 50% (approx) hydrolysis.

30 Pentapeptide solution: A standard pentapeptide solution was made to 10mg/ml in 0.1% TFA. 100 μ l was taken out and neutralised with 900 μ l 20mM Tris pH8. 10 μ l (10 μ g was taken out for dose response together with 10 μ l of the PLA₂ solution). Serial dilution was
35 carried out on 10 μ l aliquots with 20mM Tris pH 8.

Septic shock experiments:

Septic shock serum was diluted 1/100 for dose response experiments and used neat for serial dilution. Final reaction volume was always in the ratio of 10 μ l

5 serum/10 μ l Tris or pentapeptide solution.

Activity assay:

PLA₂ activity was measured using a mixed micelle phosphatidylethanolamine (PE)/sodium deoxycholate assay, modified from a method described by Seilhamer et al (1).

10 The PE substrate was prepared by dissolving freshly desiccated PE (Amersham, Bucks, England) in 2% DOC, then diluting this to 0.22 nmoles PE and 0.04% DOC per sample in assay buffer (50mM Tris-HCl, pH 8.5, 2mM calcium chloride, 150mM sodium chloride, 0.04% DOC). The sample
15 was prepared by mixing 10 μ l of the test material with 10 μ l 10mM Tris-HCl pH7.4 and leaving at 37°C for 10 minutes. The reaction was started by the addition of 25 μ l prewarmed substrate and terminated by addition of 10 μ l 100mM EDTA. The reaction mixture (30 μ l was
20 spotted and dried on silica TLC plates (Merck, Darmstadt, West Germany), and the plates were chromatographed using chloroform: methanol: acetic acid (90:10:1) as solvent. The dried plates were exposed overnight with Kodak X OMAT AR film. Radioactivity at the origin and of the liberated
25 arachidonic acid was counted and the percent hydrolysis by PLA 2 determined.

A summary of the results obtained with peptides corresponding to residues 70-74 of several Type I and Type II enzymes are set out in Table 1. These results show that
30 there is considerable species specificity in that peptides active against one species of PLA₂ were not active against the other species tested. In addition none of the peptides tested were active against PLA₂ type 1. This result indicates that inhibition by peptides from this
35 region of PLA₂ (70-74) appears to occur only on type II

- 11 -

enymes.

Peptide 5 was shown to be an active inhibitor of human Type II PLA₂, however peptides 7, 8, 9, 10, 11 and 12 were all found to be negative. This suggests that the peptide must be of a certain size to show inhibition and that inhibition will occur only with the specific sequence desired from the specific Type II enzyme being tested.

TABLE 1

Type	II	II	II	I	I
Enzyme	Syno	Crot.Dur.	Crot.Atr.	N.N.Atra	Por.Pan
Inhibitor	PLA ₂	PLA ₂	PLA ₂	PLA ₂	PLA ₂
sPLA ₂ (FLSYK)	+	-	-	-	-
Crot.Dur (WDIYR)	-	+	-	-	-
Crot.Atr (TVSYT)	-	-	+	-	-
N.N.At (FKTYS)	-	-	-	-	-
Por.Pan (TESYS)	-	-	-	-	-
sPLA ₂ -	Human Type II PLA ₂				
Crot. Dur -	<u>Crotalus</u> <u>decrissurus</u> PLA ₂				
Crot. Atr -	<u>Crotalus</u> <u>atrox</u> PLA ₂				
N.N.At -	<u>Naja</u> <u>naja</u> <u>atrox</u> PLA ₂				
Por.Pan. -	Porcine pancreatic PLA ₂				

From the above results the present inventors believe that short peptides from the 70-74th region will most likely compete with the substrate for access to the active site and give inhibitory effects. It is believed that
5 variation of the length of the peptides present in these regions may result in a optimisation of the inhibition.

The pentapeptide apparently possesses helical structure (approximately one and a half turns). Since the helical structures are stabilised by hydrogen bonds between
10 the C=O of one residue and NH of the fourth residue along the chain, the structure of the pentapeptide may be somewhat unstable and be more sensitive to the environment than a longer helical molecule. On the other hand, it would be expected that the range of sizes that is
15 effective will be limited because of the limited access to the active site of PLA₂.

It is believed that the usual interchange of a hydrophobic residue e.g. Leu to Ile, Ser to Thr could also maintain the inhibitory effect. That is, amino acid
20 residues alike in either charge or hydrophobicity could possibly be interchanged with those in the models without destroying the specific interaction of the two regions. Since helix-helix interactions are possibly the cause of the inhibitory action, small increases in the length of
25 the peptides could stabilise this structure.

The results obtained in these studies also suggest that monoclonal antibodies could be raised against epitopes containing one or both of the peptide regions to effect a similar result on the PLA₂ activity. Such
30 monoclonal antibodies could be produced using standard techniques well known in the art.

As will be apparent to those skilled in the art the principle of the present invention will also have application for the inhibition of the activity of enzymes
35 other than PLA₂ eg. the neuraminadase enzyme of the

- 14 -

influenza virus or neuropeptide Y. It is envisaged that as biological active proteins in general, possess an active conformation which is stabilized by interaction with the surrounding chain of amino acids, that peptides
5 adjacent to, and capable of interaction with the residues within the active site will inhibit the activity of the enzyme. It is intended that such other peptides are included within the scope of the present invention.

It will be appreciated by persons skilled in the art
10 that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as
15 illustrative and not restrictive.

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- 16 -

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

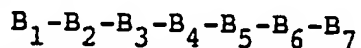
1. A compound which inhibits the enzymatic activity of Type II phospholipases A_2 , the compound being characterized in that it interacts with the N-terminal amino acid sequence of the phospholipase A_2 such that the channel into which the phospholipid diffuses prior to catalytic cleavage is either blocked or destabilized.
2. A compound as claimed in claim 1 in which the PLA_2 is human PLA_2 .
3. A compound as claimed in claim 1 or claim 5 in which the compound is a peptide.
4. A compound as claimed in claim 3 in which the peptide is a pentapeptide.
5. A linear or cyclic peptide of at least 5 residues which inhibits the enzymatic activity of human synovial PLA_2 , the peptide having the following formula:-
$$A_1-A_2-A_3-A_4-A_5-A_6-A_7$$

in which A_1 is H or one of two naturally occurring amino acids
- 20 A_2 is F or Y or W or absent
 A_3 is L or V or I or M
 A_4 is S or T
 A_5 is Y or F or W
 A_6 is K or R or H or absent
- 25 A_7 is OH or one or two naturally occurring amino acids.
6. A peptide as claimed in claim 1 in which the peptide is a pentapeptide.
7. A peptide as claimed in claim 1 or claim 2 in which
- 30 A_1 is H and A_7 is OH.
8. A peptide as claimed in any claims 3 to 7 in which the peptide is FLSYK or KFLSY.
9. A peptide of 5 or 6 residues which inhibits the enzymatic activity of a phospholipase A_2 , the peptide
- 35 having an amino acid sequence corresponding to a sequence

- 17 -

selected from the region of residues 69-75 of the phospholipase A₂.

10. A peptide as claimed in claim 9 in which the peptide is a pentapeptide and has an amino acid sequence
5 corresponding to the sequence from residue 69-73 or 70-74 of the phospholipase A₂.
11. A peptide as claimed in claim 9 or claim 10 in which the phospholipase A₂ is human phospholipase A₂.
12. A linear or cyclic peptide of at least 5 residues
10 which inhibits the enzymatic activity of crotalus durissus PLA₂, the peptide having the following formula:-



in which B₁ is H or one of two naturally occurring amino acids

- 15 B₂ is W or F or Y or absent

B₃ is D or E

B₄ is I or V or L or M

B₅ is Y or F or W

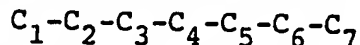
B₆ is R or K or H or absent

- 20 B₇ is OH or one or two naturally occurring amino acids.

13. A peptide as claimed in claim 12 in which B₁ is H and B₇ is OH.

14. A peptide as claimed in claim 12 or claim 13 in which
25 the peptide is WDIYR.

15. A linear or cyclic peptide of at least 5 residues which inhibits the enzymatic activity of Crotalus atrox PLA₂, the peptide having the following formula:



- 30 in which C₁ is H or one of two naturally occurring amino acids

C₂ is T or S or absent

C₃ is V or I or L or M

C₄ is S or T

- 35 C₅ is Y or F or W

- 18 -

C₆ is T or S or absent

C₇ is OH or one or two naturally occurring amino acids.

16. A peptide as claimed in claim 15 in which C₁ is H
5 and C₇ is OH.
17. A peptide as claimed in claim 15 or claim 16 in which the peptide is TVTSYT.
18. A composition for use in treating the subject suffering from rheumatoid arthritis, septic shock and/or
10 inflammatory disease, the composition comprising a therapeutically effective amount of the peptide as claimed in any one of claims 1 to 11 and a pharmaceutically acceptable sterile carrier.
19. A method of treating rheumatoid arthritis, septic
15 shock and/or inflammatory disease in a subject comprising administering to the subject the composition of claim 18.

1/9

FIG. 1

Exon 2:	Type	1	10	20	30	40	
porcine	I	<u>ALWOFRSMIKCAIPGSHPLMDFNNYGCYCGLGGSGTPVDELDR</u>					
rat	I	<u>AVWOFRNMIKCTIPGSDPFREYNNYGCYCGLGGSGTPVDDLDR</u>					
human	I	<u>AVWOFRKMIKCVIPGSDPFLEYNNYGCYCGLGGSGTPVDELDK</u>					
		* * * * *					
human	IIA	<u>NLVNFRMIK-LTTGKEAALS</u> <u>YGFGCHCGVGGRGSPKDATDR</u>					
rat	IIA	<u>SLLEFGOMIL-FKTGKRADVSYGFGCHCGVGGRGSPKDATDE</u>					
porcine	IIA	<u>DLNFRKMIK-LKTGKAPVPNYAFYGCYCGLGGKSPKDATD?</u>					
rabbit	IIA	<u>HLLDFRKMIR-YTTGKEATTSYGAYGCHCGVGGRGAPK?A</u>					
<hr/>							
Exon 3:		44	50	60	70	80	85
porcine	I	<u>CCETHDNCYRDAKNLDSCKELVDN</u> <u>PYTESYSYSCSNTEITCN</u>					
rat	I	<u>CCOTHDHCYNQAKKLESCKELIDNPYTNTYSYKCSGNVITCS</u>					
human	I	<u>CCOTHDNCYDOAKKLDSCKFLLDNPYTH</u> <u>TYSYSCSGSAITCS</u>					
		**					
human	IIA	<u>CCVTHDCCYKRLEKR-GC-----G</u> <u>TKFELSYKESNSGSRITC-</u>					
rat	IIA	<u>CCVTHECCYNRLEKS-GC-----G</u> <u>TKELTYKESYRGGQISCS</u>					
porcine	IIA	<u>CCAAH</u>					
rabbit	IIA	<u>KELSYKESMK</u>					
<hr/>							
Exon 4:		86	90	100	110	120	130
porcine	I	<u>SKNNACEAFICNCDRNAAICFSKAPYNKEHK-NLDTKKYC</u>					
rat	I	<u>DKNNDCESEFICNCDROAAICFSKVPYNKEYK-DLDTKKHC</u>					
human	I	<u>SKNKECEAFICNCDRNAAICFSKAPYNKAHK-NLDTKKYCQS</u>					
		**					
human	IIA	<u>AKODSCRSOLCECDKAAATCFARNKTTYNKKYQYYSNKHCRGSTPRC</u>					
rat	IIA	<u>TNODSCRKOLCQCDKAAAEFCFSRNKKSYS</u> <u>SLKYQFYPNKFCK??TPSC</u>					
rabbit	IIA	<u>KAAAACE</u> <u>QFYPANRCGRPPSC</u>					

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2/9

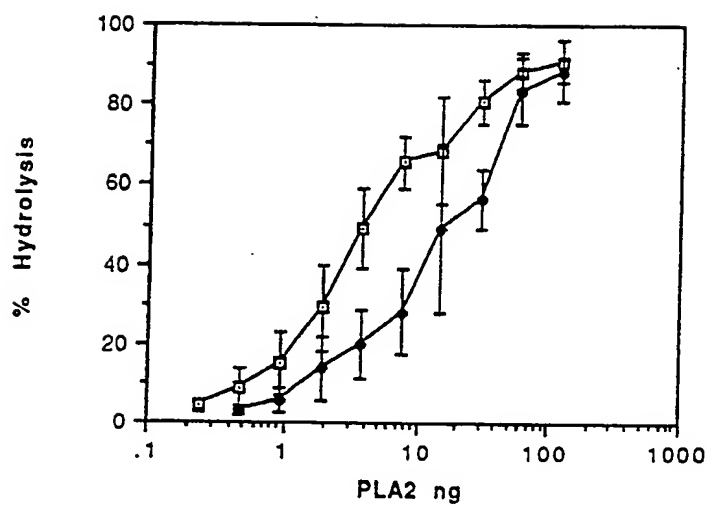


FIG. 2a.

3/9

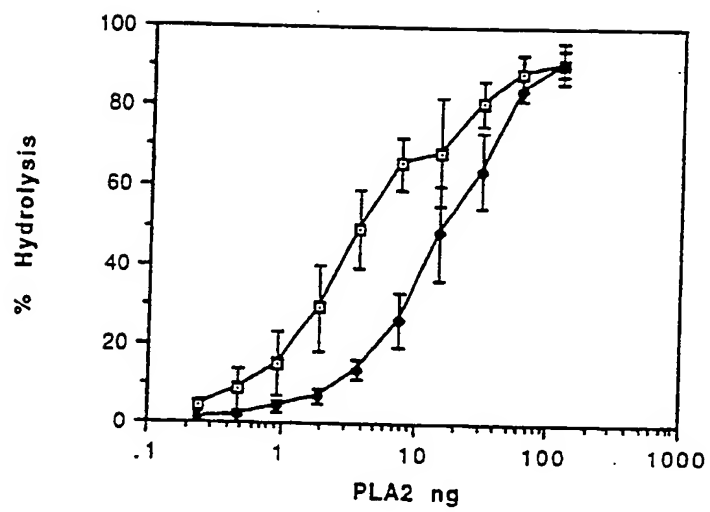


FIG. 2b.

4/9

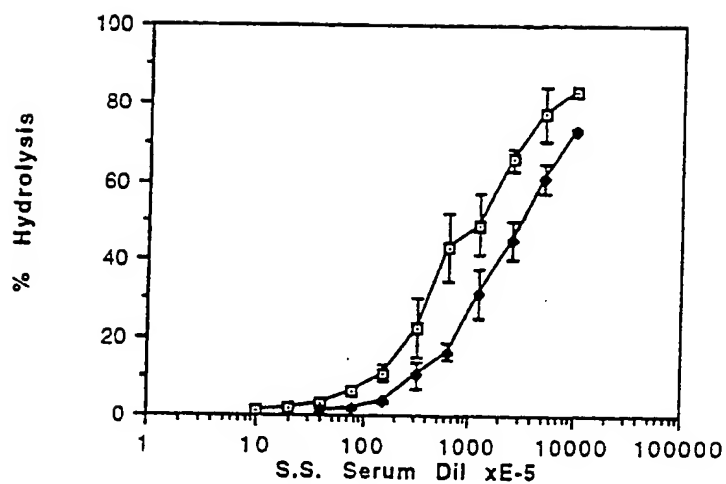


FIG. 2c.

5/9

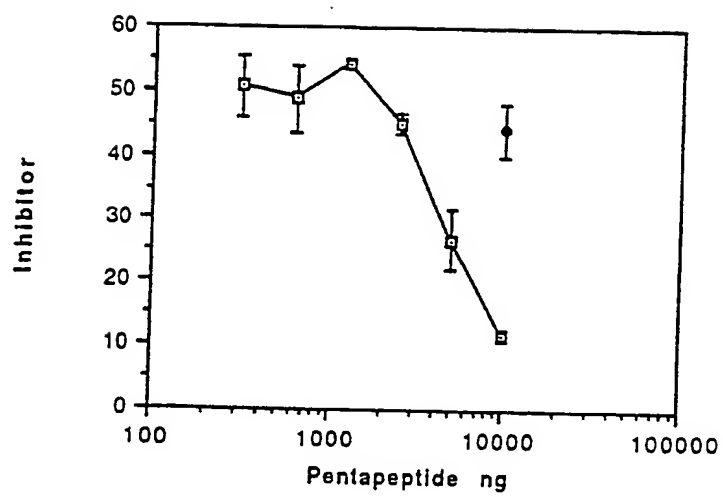


FIG. 3a.

6/9

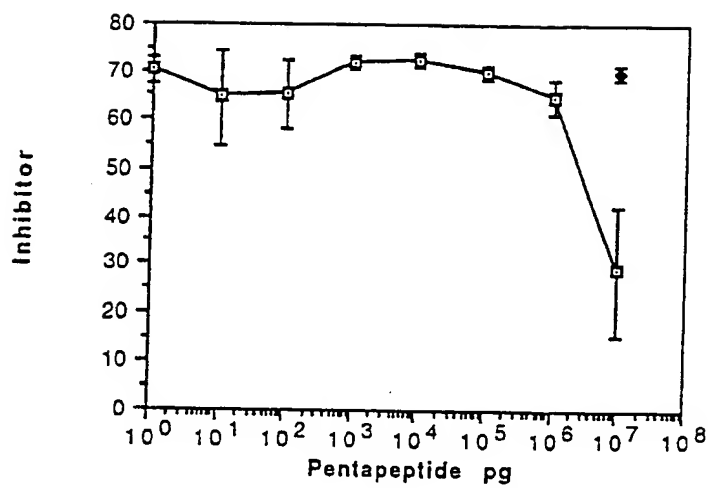


FIG. 3b.

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7/9

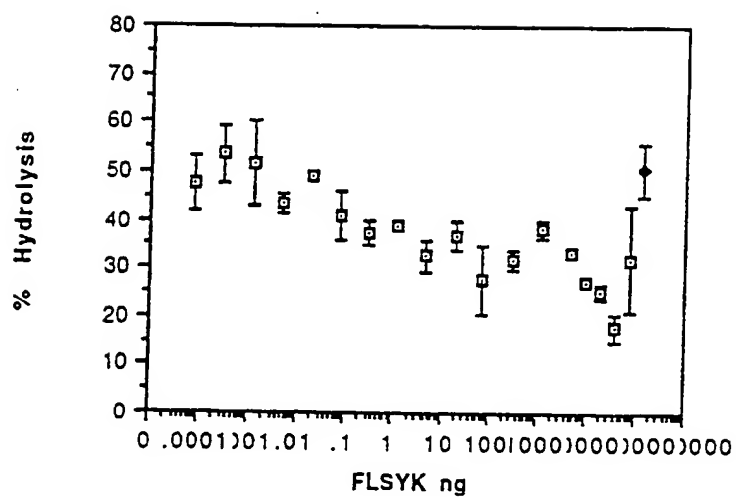


FIG. 4a

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8/9

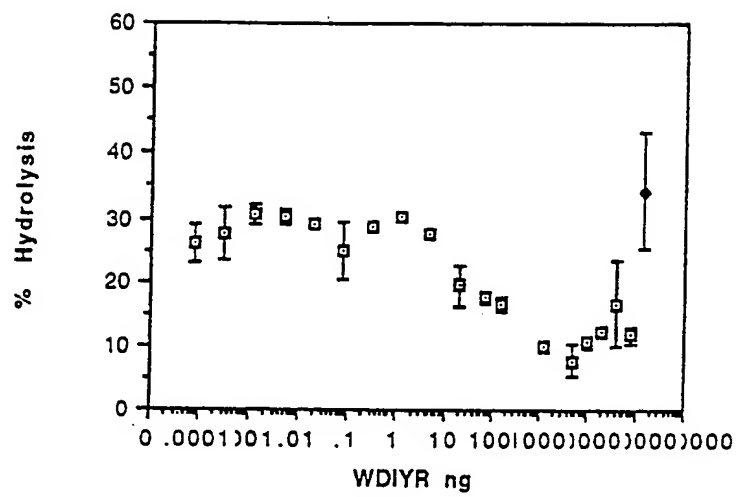


FIG. 4b.

9/9

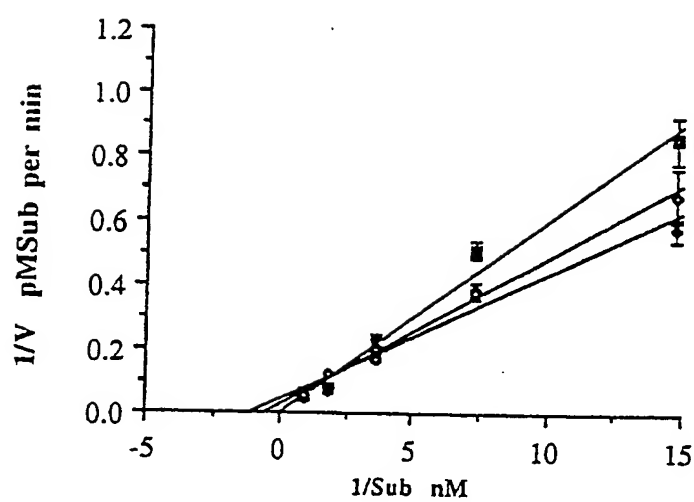


FIG. 5.

A. CLASSIFICATION OF SUBJECT MATTERInt. CL⁵ C07K 007/06, C07K 007/64, A61K 037/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: WPAT: see below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)

WPAT (PHOSPHOLIPASE: OR PLA2) & (INHIBIT: OR ANTAGONIST:)

Chemical Abstracts: STN data base peptide sequence

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	AU-B-50307/85 (583553) (Zaidan Hosin Biseibutsu Kagaku Kenku Kai) 12 June 1986. See page 3 line 22-page 5 line 3, Table 2	
A	AU-B-15452/88 (610579) (American Home Products Corp) 22 September 1988. See page 3 line 17-page 7 line 15, page 8 line 24, claims	
A	Patent Abstracts of Japan No J 63-255298(A) (Yamansuchi Pharm Co Ltd) 21 October 1988. See abstract	

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.*** Special categories of cited documents :**

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search
15 October 1992 (15.10.92)

Date of mailing of the international search report

20 Oct 1992 (20.10.92)

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
A	AU-A-15263/88 (Hoechst A G) 3 November 1988. See page 1a line 26-page 2 line 4, Examples, Claims	
A	AU-B-28127/89 (623620) (The United States of America as represented by The Secretary, US Department of Commerce) 15 June 1989. See page 3 line 25-page 4 line 14 and claims	
A	EP-A 327334 (Kyowa Hakko Kogyo Co Ltd) 9 August 1989. See column 1 lines 1-59, Examples, Claims	
A	Chemical Abstracts, volume 116(5): 35305 & BOUCHIER et al "Analysis of cDNAs encoding the two subunits of crotoxin, a phospholipase A2 neurotoxin from rattlesnake venom:- BIOCHIM BIOPHYS ACTA, 1088(3) 401-8	
A	Chemical Abstracts volume 112(3): 17274 & Seilhamer et al, "Cloning and Recombinant expression of phospholipase A2 present in rheumatoid arthritic synovial fluid" J Biol Chem, 264(10) 5335-8	
A	Chemical Abstracts volume III(25): 227907 & Kramer et al. "Structure and properties of a human non-pancreatic phospholipase A2" J BIOL CHEM 246(10) 5768-75	

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
AU-B- 50307/85	HU-A- 40707 EP,A, 192828 ZA-A- 8509315	DK,A, 5647/85 US-A- 4742155	JP-A- 61134398 ES,A, 8701197
AU-B- 15452/88	EP-A- 305492 WO-A- 8806885	US-A- 4792555	GB-A 2202534
JP-A- 63255298	NIL		
AU-A- 15263/88	DK-A- 2330/88 PT-A- 87350 IL-A- 86195	JP-A-63284197 EP-A- 288965	DE-A- 3714277 ZA-A- 8803033
AU-B- 28127/89	EP-A- 397679	WO-A-8905147	
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END OF ANNEX			